In-Process Test		Comments
Liver Acceptance criteria Components Release	Receive Liver in Viaspan (4°C)	Donor Screening & Serology Testing provided by OPO. Confirmatory testing by contract lab
Sample Viaspan for sterility testing (Bactec)	Transfer to a class 100 hood, weigh Liver	
	Aseptic technique	
	Antibiotic wash, Rinse	
	Cannulate and biopsy	
	Perfuse with warm chelation buffer	120-260 mL/min 15 min
	Digestion with Liberase buffer	120-200 mL/min 28 - 37°C 30 min
	Turn off heat	
	Perfuse with cold, serum-containing collection buffer	5 min
	Mechanical serration in cold, serum-containing collection buffer	≤ 20 min
	Filter using prefilter and presterilized stackable stainless steel sieves : 1000, 500, 250, and 150 μm	
Crude Cell Suspension: • Viability, • Total cell count • Cell concentration • Yield /g tissue • Sterility (Bactec)	Crude Cell Suspension in Blood bag	4°C

Figure 17A

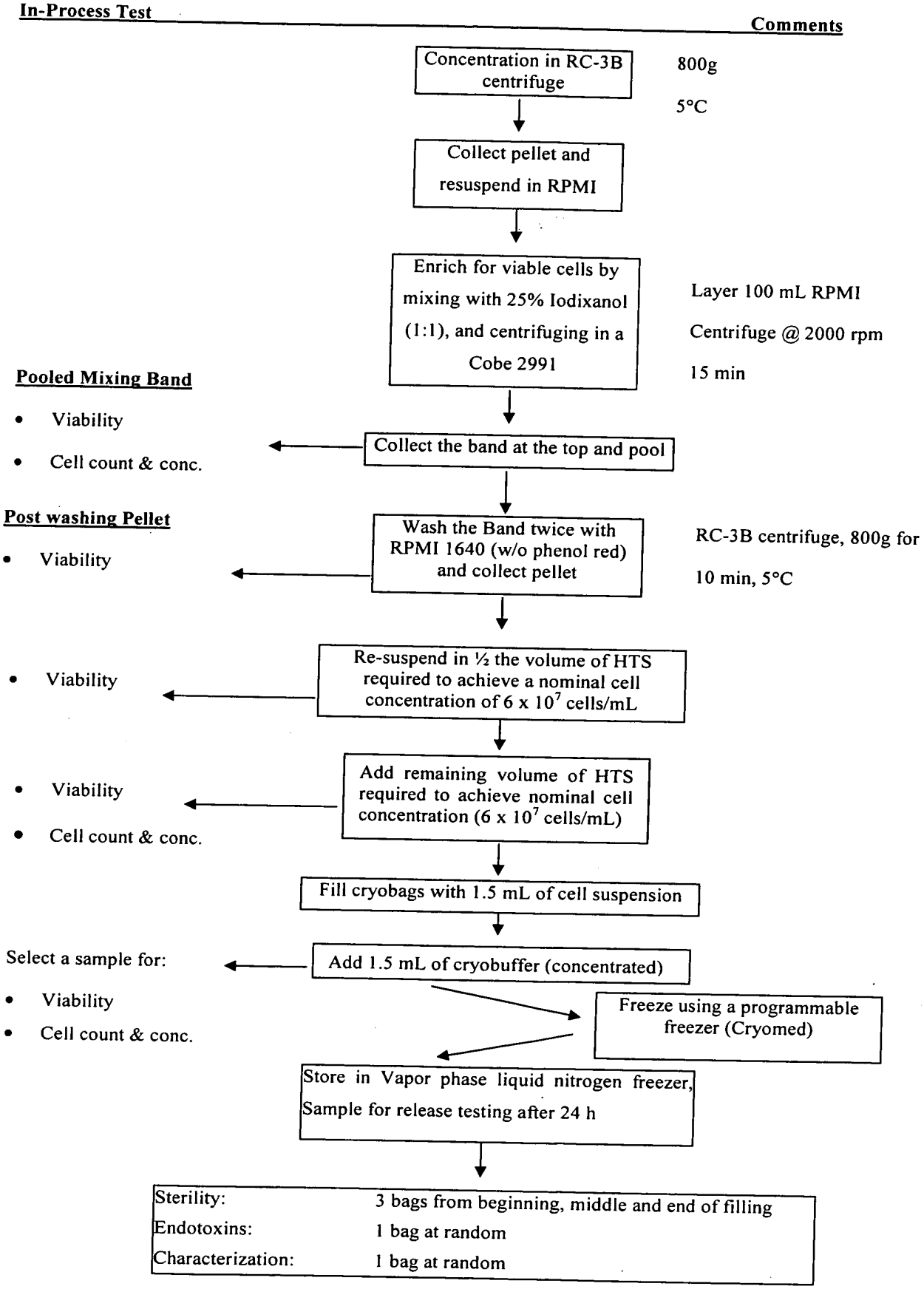
In-Process Test
Comments
Concentration in RC-3B centrifuge
800g
5°C
Collect pellet and resuspend in RPMI
Enrich for viable cells by mixing with 25% Iodixanol (1:1), and centrifuging in a Cobe 2991
Layer 100 mL RPMI
Centrifuge @ 2000 rpm
15 min
Pooled Mixing Band
• Viability
• Cell count & conc.
Collect the band at the top and pool
Post washing Pellet
• Viability
Wash the Band twice with RPMI 1640 (w/o phenol red) and collect pellet
RC-3B centrifuge, 800g for 10 min, 5°C
• Viability
Re-suspend in ½ the volume of HTS required to achieve a nominal cell concentration of 6 x 10^7 cells/mL
• Viability
• Cell count & conc.
Add remaining volume of HTS required to achieve nominal cell concentration (6 x 10^7 cells/mL)
Fill cryobags with 1.5 mL of cell suspension
Select a sample for:
• Viability
• Cell count & conc.
Add 1.5 mL of cryobuffer (concentrated)
Freeze using a programmable freezer (Cryomed)
Store in Vapor phase liquid nitrogen freezer, Sample for release testing after 24 h
Sterility: 3 bags from beginning, middle and end of filling
Endotoxins: 1 bag at random
Characterization: 1 bag at random

Figure 17B